IN THE SPECIFICATION:
(add underlining from the original...)
(Keep Seq ID No.)
On page 4, please amend the paragraph beginning at line 5 as follows:

The present invention describes in particular the isolation and the characterization of the gr1A and gr1B genes. These genes have been cloned, sequenced and expressed in E. coli, and their enzymatic activity has been characterized. They were isolated from a Staphylococcus aureus genomic DNA library. From the gr1AB nucleic acid sequence [[(SEQ ID No. 1)]] (SEQ ID NO:1 and SEQ ID NO:2), two open frames, corresponding to the gr1B and gr1A genes respectively, have been identified. The gr1A and gr1B genes have been sequenced in [[SEQ ID No. 2 and SEQ ID No. 3]] (SEQ ID NO:4) and (SEQ ID NO:6), respectively.

On page 4, please amend the paragraph beginning at line 18 as follows:

(a) all or part of the gr1A [[(SEQ ID No. 2)]] (SEQ ID NO:4) or gr1B [[(SEQ ID No. 3)]] (SEQ ID NO:6) genes,

On page 5, please amend the paragraph beginning at line 26 as follows:

Still more preferably, the subject of the invention is the nucleotide sequences represented by the gr1A [[(SEQ ID No. 2)]] (SEQ ID NO:4) and gr1B [[(SEQ ID No. 3)]] (SEQ ID NO:6) genes.

On page 6, please amend the paragraph beginning at line 1 as follows:

It also relates to any gr1A gene having a mutation leading to a resistance to molecules of the quinoline and more particularly of the fluoroquinolone family. As a

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

representative of these mutated genes, there may be mentioned more particularly the gr1A gene having a base change from C to A at position 2270 of [[SEQ ID No. 2]] SEQ ID NO:4. The resulting gene is termed gr1A $_{(C\text{-}2270A)}$. This mutation leads to substitution of the Ser-80 residue with Tyr in the Gr1A protein. The resulting protein will be designated by Gr1A $_{(Ser\text{-}80\ Tyr)}$.

On page 6, please amend the paragraph beginning at line 11 as follows:

Another subject of the present invention relates to a recombinant DNA comprising at least one nucleotide sequence encoding a subunit of topoisomerase IV of Staphylococcus aureus. More preferably, it is a recombinant DNA comprising at least one nucleotide sequence as defined above in (a), (b), and (c) and more particularly the gene gr1A [[(SEQ ID No. 2)]] (SEQ ID NO:4) gr1A $_{(C\text{-}2270A)}$ and/or the gene gr1B [[(SEQ ID No. 3)]] (SEQ ID NO:6).

On page 6, please amend the paragraph beginning at line 23, and continuing through page 7, line 20 as follows:

Another subject of the invention relates to the polypeptides resulting from the expression of the nucleotide sequences as defined above. More particularly, the present invention relates to the polypeptides comprising all or part of the polypeptides Gr1A [[(SEQ ID No. 2)]] (SEQ ID NO:3) or Gr1B [[(SEQ ID No. 3)]] (SEQ ID NO:5) or of their derivatives. For the purposes of the present invention, the term derivative designates any molecule obtained by modification of the genetic and/or chemical nature of the peptide sequence. Modification of the genetics and/or chemical nature may be

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

understood to mean any mutation, substitution, deletion, addition, and/or modification of one or more residues. Such derivatives may be generated for different purposes, such as especially that of increasing the affinity of the peptide for its substrate(s), that of enhancing its production levels, that of increasing its resistance to proteases, that of increasing and/or of modifying its activity, or that of conferring new biological properties on it. Among the derivatives resulting from an addition, there may be mentioned, for example, the chimeric polypeptides containing an additional heterologous part attached to one end. The term derivative also comprises the polypeptides homologous to the polypeptides described in the present invention, derived from other cellular sources.

On page 7, please amend the paragraph beginning at line 21 as follows:

Preferably, they are the polypeptides Gr1A [[(SEQ ID No. 2)]] (SEQ ID NO:3), Gr1B [[(SEQ ID No. 3)]] (SEQ ID NO:5) and $Gr1A_{(Ser\text{-}80\ Tyr)}$.

On page 8, please amend the paragraph beginning at line 23 as follows:

The invention also relates to an isolated topoisomerase IV capable of being obtained from the expression of all or part of the gr1A gene [[(SEQ ID No. 2)]] (SEQ ID NO:4) and of all or part of the gr1B gene [[(SEQ ID No. 3)]] (SEQ ID NO:6) or of their respective derivatives.

On page 9, please amend the paragraph beginning at line 7 as follows:

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

More preferably, it is an isolated topoisomerase IV derived from the expression of all or part of the gr1A gene [[(SEQ ID No. 2)]] (SEQ ID NO:4) or of all or part of the gr1B gene [[(SEQ ID No. 3)]] (SEQ ID NO:6).

On page 12, please amend the paragraph beginning at line 4, and continuing through page 13, line 9, as follows:

This example describes the production of DNA fragments of Staphylococcus aureus which are inside the gr1A and gr1B genes. These fragments were obtained after PCR amplification carried out at 50°C with genomic DNA of the Staphylococcus aureus strain RN 4220 (Novick, 1990) and of the degenerate oligonucleotides corresponding to the amino acids conserved in the N-terminal regions of the subunits of GyrA of E. coli and B. subtilis and ParC of E. coli or of the subunits GyrB of E. coli and B. subtilis and ParE of E. coli. More specifically, the sense oligonucleotides 2137 and antisense oligonucleotides 2135 made it possible to amplify fragments of 255 bp which can encode 85 amino acids which would correspond to positions 39 to 124 on the E. coli GyrA sequence; the sequence of the sense oligonucleotide 2137 is 5'-GCGCGAATTCGATGG(A,T)(C,T)T(A,T)AAACC(A,T)GT(A,T)CA-3' [[(SEQ ID No. 4)]] (SEQ ID No. 7) and that of the antisense 2135 is 5'-CGCGAAGCTTTTC(T,A)GTATA(A,T)C(T,G)CAT(A,T)GC(A,T)GC-3' [[(SEQ ID No. 5)]] (SEQ ID No. 8). The oligonucleotides 2144 and 2138 led to the amplification of 1 kb fragments which can encode 333 amino acids which would correspond to positions 98 to 430 on the E. coli GyrB sequence; the sequence of the sense oligonucleotide 2144 is 5'-GCGCGAATTCT(T,A)CATGC(A,T)GG(T,A)GG(T,A)AAATT-3' [[(SEQ ID No. 6)]]

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

(SEQ ID No. 9), and that of the antisense 2138 is 5'-CGCGAAGCTT(T,A)CC(T,A)CC(T,A)GC(T,A)GAATC(T,A)CCTTC-3' [[(SEQ ID No. 7)]] (SEQ ID No. 10). The fragments were cloned and a total of 40 clones were analysed by sequencing their insert. The sequence of the oligonucleotides used for the PCR was found for 31 clones out of 40; among the 31 clones, 20 have a sequence which is inside the gyrA or gyrB gene of S. aureus; the other 11 clones contain either a fragment A of 255 bp or a fragment B of 1 kb.

On page 14, please amend the paragraph beginning at line 19, and continuing through page 15, line 19, as follows:

The inserts contained in the recombinant phages pXL2321, pXL2322 and pXL2324 were sequenced on both strands with the aid of the universal primer or of internal oligonucleotides using the Sanger method. The nucleic sequence gr1AB [[(SEQ ID No. 1)]] (SEQ ID NO:1 and SEQ ID NO:2) of 4565 bp was analysed with the programme by Staden et al., 1982, in order to identify the coding sequences with the aid of a codon usage table for S. aureus. Only two open reading frames ORF1 (positions 41 to 2029) and ORF2 (positions 2032 to 4431) were thus determined. [On SEQ ID No. 1, the coding strand is the 5' => 3' top strand] In SEQ ID NO:1, which is the coding strand, the open frame ORF1 starts arbitrarily at ATG position 41 but it can also start at TTG position 17 or 35, this codon being already described as an initiation codon in S. aureus; the stop codon of ORF1 overlaps with the initiation codon GTG of ORF2, which is characteristic of a translational coupling (Normark et al., 1983); such a coupling has, for example, been described for the gyrA and gyrB genes of Haloferax sp. (Holmes

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

et al., 1991). These open frames have a percentage of GC of 34.5% which is a value in agreement with the values described for the S. aureus DNA in the literature (Novick, 1990). Moreover, the B fragment is identical to the sequence described on [SEQ ID No. 1] SEQ ID No:1 from position 333 to position 1348 in ORF1 and the fragment A is identical to the sequence of [SEQ ID No. 1] SEQ ID No:1 from position 2137 to position 2394 in ORF2. From the nucleotide sequence, a restriction map is produced with enzymes which cut least frequently, see Figure 1.

On page 16, please amend the paragraph beginning at line 6 as follows:

This example describes computer analysis of the sequence of the gr1A and gr1B genes of Staphylococcus aureus carried out using the sequence data presented in Example 2. The gr1B gene encodes a Gr1B protein of 663 amino acids (molecular weight 74,318), and the gr1A gene encodes a Gr1A protein of 800 amino acids (molecular weight 91,040). The coding parts of the gr1B and gr1A genes, the sequences of the Gr1B and Gr1A proteins are presented in [[SEQ ID No. 3]] SEQ ID NO:5 and [[SEQ ID No. 2]] SEQ ID NO:3 respectively and the properties of each of these proteins (amino acid composition, isoelectric point, polarity index) are presented in Tables 1 and 2 below.

On page 19, please amend the paragraph beginning at line 27, and continuing through page 20, line 14, as follows:

Multiple alignments between the type II bacterial topoisomerases, performed with the CLUSTAL programme of Higgins et al., 1988, show numerous conserved regions

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

between the sequences of the various B, Gr1B and ParE subunits and in the N-terminal part of the sequence of the A, Gr1A, and ParC subunits. The residues conserved in the N-terminal region of the B subunits of these proteins are in fact the residues involved in the binding of ATP and identified from X-ray crystallization data with the E. coli GyrB (Wigley et al., 1991). The residues conserved in the N-terminal region of the A subunits of these proteins are either the residues AAMRYTE [[(SEQ ID No. 8)]] (SEQ ID NO:11) close to the active site of gyrase Tyr-122, identified on the E. coli GyrA (Horowitz et al, 1987) or the residues YHPHGDS [[(SEQ ID No. 9)]] (SEQ ID NO:12) modified in the strains resistant to fluoroquinolones (Hooper et al., 1993).

On page 23, please amend the paragraph beginning at line 13, and continuing through page 24, line 9, as follows:

The genomic DNA of eight clinical strains of S. aureus and of a laboratory strain was prepared and used to amplify at 42°C by PCR: i) the first 500 base pairs of gyrA using the sense oligonucleotide 5'-GGCGGATCCCATATGGCTGAATTACCTCA-3' [[(SEQ ID No. 10)]] (SEQ ID NO:13) and the antisense oligonucleotide 5'-GGCGGAATTCGACGGCTCTCTTTCATTAC-3' [[(SEQ ID No. 11)]] (SEQ ID NO:14); ii) and the first 800 base pairs of gr1A using the sense oligonucleotide 5'-GGCCGGATCCCATATGAGTGAAATAATTCAAGATT-3' [[(SEQ ID No. 12)]] (SEQ ID NO:15) and the antisense oligonucleotide 5'-GGCCGAATTCTAATAATTAACTGTTTACGTCC-3' [[(SEQ ID No. 13)]] (SEQ ID NO:16). Each amplified fragment was cloned in the phage M13mp18 and the sequence of the first 300 base pairs of each of the genes was read on 2 clones. They gyrA

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

sequence is identical to that published by Magerrison and that of gr1A to that described in [SEQ ID No. 1] SEQ ID NO:1, with the exception of the mutations presented in Table 4. The mutations in gyrA exist with the strains highly resistant to fluoroquinolones (SA4, SA5, SA6, SA35, SA42 and SA47; MIC for ciprofloxacin > 16 mg/l); these mutations are a base change which leads to changes in the amino acids Ser-84 or Ser-85 or Glu-88. A mutation in gr1A exists with all the strains resistant to fluoroquinolones and corresponds to the changing of the residue Ser-80 to Phe or Tyr.

On page 25, please amend the paragraph beginning at line 22, and continuing through page 26, line 27, as follows:

This example describes the production of the DNA fragment which is inside gr1A of an S. aureus strain, SA 2, resistant to fluoroquinolones. The gr1A fragment contains a base change from C to A at position 2270 of the wild-type gene (Fig. 1). This mutation leads to a substitution of the residue Ser-80 to Tyr in the Gr1A protein. It has been shown that a substitution of the residue Ser-80 to Phe or Tyr exists with all the strains weakly resistant to fluoroquinolones (Example 4). The fragment which is inside gr1A was obtained after PCR amplification carried out at 50°C with the genomic DNA of the SA2 strain and of the oligonucleotides 3358 and 3357 which correspond to position 2036 and 3435 respectively on the sequence of gr1A. More specifically, the sense oligonucleotide 3358 [[(SEQ ID No. 12)]] (SEQ ID NO:15) (Example 4) and the antisense oligonucleotide 3357 made it possible to amplify a fragment of 1399 base pairs; the sequence of the antisense oligonucleotide 3357 is 5'-GGCCGAGCTCCAATTCTTCTTTTATGACATTC-3' [[(SEQ ID No. 14)]] (SEQ ID

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

NO:17). The oligonucleotide 3358 was also used to introduce, by mutagenesis, a sequence CATATG, in place of the GTG initiation codon in order to create an NdeI site. The amplified gr1A fragment was cloned into the BamHI/SstI cloning sites of pUC18 (Boehringer Mannheim), and 6 clones containing this plasmid, pXL2692, were analysed after sequencing their insert. In all cases, a sequence CATATG was introduced in place of the CTG initiation codon, and the point mutation at position 2270 of gr1A (C>A) was again found.

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com